cell monolayer, thus reducing undesirable extracellular background fluorescence; it provides data in real-time, and can also provide kinetic data (*i.e.*, readings at a multitude of timepoints); it has the ability to simultaneously stimulate and read all 96 wells of a 96-well microplate; it provides for precise control of temperature and humidity of samples during analysis; it includes an integrated state-of-the-art 96-well pipettor, which uses dispensible tips to eliminate carryover between experiments, that can be used to aspirate, dispense and mix precise volumes of fluids from microplates; and, in the case of the FLIPR^{384} instrument, it can be adapted to run sample assays in a robotic or semi-robotic fashion, thus providing for analysis of large numbers of samples in shortest amount of time (*e.g.*, up to about a hundred 96-well microplates per day).

In the Claims:

Please cancel claims 22-42 and 60-63 without prejudice.

Please amend claims 1, 19, 20, 21, 43, 64, 65-68, 71-74, 78-79, 80, 81 and 93 to read as follows:

1. (Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

19. (Amended) A method of identifying an agent that uncouples oxidative phosphorylation from ATP production, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity;;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point prior to a contacting step in the presence of the agent, compared to the level of calcium in the cytosol prior to a contacting step in the absence of the agent, indicates an agent that uncouples oxidative phosphorylation from ATP production.

20. (Amended) A method of identifying an agent that that is a respiratory inhibitor, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point prior to a contacting step in the presence of the agent, compared to the level of calcium in the cytosol prior to a contacting step in the absence of the agent, indicates an agent that is a respiratory inhibitor.

21. (Amended) A method of identifying an agent that alters a mitochondrial calcium uniporter, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point following a contacting step in the presence of the agent, compared to the level of calcium in the cytosol following a contacting step in the absence of the agent, indicates that the agent alters a mitochondrial calcium uniporter.

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43. (Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing a mitochondrion, cytosol and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, and wherein the calcium indicator molecule is membrane permeable and capable of generating a detectable signal that is proportional to the level of calcium in the cytosol, with

(ii) a calcium ionophore, under conditions and for a time sufficient to increase calcium levels within the cell;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of the candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

64. (Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a permeabilized cell depleted of cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cell;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

65. (Amended) The method of claim 64 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the mitochondrion.

A7

66. (Amended) The method of claim 64 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium outside of the mitochondrion.

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67. (Amended) The method of claim 64 wherein the step of contacting is repeated at least once.

68. (Amended) The method of claim 64 wherein the sample contains at least one compound that alters intracellular distribution of a calcium cation.

71. (Amended) The method of claim 64 wherein the source of calcium cations is exogenous to the cell.

72. (Amended) The method of claim 64 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

A8

73. (Amended) The method of claim 64 wherein the cell comprises at least one polypeptide that is a Bcl-2 family member.

74. (Amended) The method of claim 64 wherein the cell expresses a gene encoding a polypeptide that regulates cytosolic calcium.

A9

78. (Amended) The method of claim 64 wherein the cell adheres to a solid substrate.

79. (Amended) The method of claim 64 wherein the cell is a non-adherent cell.

80. (Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising one or more isolated mitochondria and a calcium indicator molecule in a medium, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the biological sample;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

81. (Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting

(i) a biological sample comprising one or more isolated mitochondria and a calcium indicator molecule in a medium, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,